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Genotypes of Hepatitis C Virus in Nigeria

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A pilot survey of hepatitis C virus (HCV) infection in Nigeria was carried out on healthy adult blood donors and children of preschool age. Sixteen of 200 (8%) donors were positive for antibodies using a second generation enzyme-linked immunosorbent assay (ELISA) but all of the children were negative. Supplementary testing of the ELISA-positives using a recombinant immunoblot assay (RIBA-2) confirmed the presence of antibody in four and two others were indeterminate. Four of the anti-HCV-positive sera and one found positive by ELISA but which was negative by RIBA-2 were found to be positive for HCV RNA using reverse transcriptase-polymerase chain reaction (RT-PCR) and primers specific for the 5′ untranslated region (5′UTR) of the HCV genome. The NS5 and core regions also were amplified and the PCR products from all three regions were sequenced. Sequences from the 5′UTR could be divided into two groups: one group comprised three isolates with greater than 95% sequence identity with published sequences of genotype 1 and the other comprised two isolates with greater than 93% sequence identity with genotype 4. Analysis of three sequences amplified from the NS5 region confirmed this assignment to genotypes 1 and 4. Pairwise comparisons of the NS5 region sequences with representatives of 1a, 1b, 1c (for the first group) and 4a–4h (for the second group) show the first group to include subtypes classifiable as 1a and a novel sequence and the second group to include a novel sequence within genotype 4. Sequence analysis of the core region was consistent with this interpretation. These data confirm the presence of at least two major HCV genotypes in Nigeria (genotypes 1 and 4) and we report two novel sequences which have been designated provisionally as genotypes 1d and 4i.

KEY WORDS: ELISA, RIBA, sequence analysis, nucleotide homology

INTRODUCTION

Hepatitis C virus (HCV) has been identified as the etiological agent responsible for most cases of parenterally transmitted non-A, non-B hepatitis [Choo et al., 1990; Kuo et al., 1989]. The HCV genome is a positive polarity, single-stranded RNA of approximately 9.4 kilobases (kb) in length [Choo et al., 1991]. The genome consists of three regions, a 5′ untranslated region (5′UTR) which comprises approximately 341 nucleotides [Han et al., 1991], a single continuous open reading frame encoding a polyprotein of 3,010 amino acid residues [Choo et al., 1991], and a short 3′UTR [Takamizawa et al., 1991]. The 5′UTR and region encoding the nucleocapsid protein (C) are relatively conserved [Takeuchi et al., 1990; Han et al., 1991; Bukh et al., 1992, 1994], while regions encoding envelope glycoproteins 1 (E1) and 2 (E2) are highly variable and a hypervariable region (HVR-1) is present in the N-terminus of E2 [Weiner et al., 1991]. The remainder of the open reading frame encodes non-structural polypeptides and is moderately variable.

The prevalence of HCV infection generally is low in Western countries, but a higher prevalence has been reported in some parts of southern and eastern Europe [Esteban et al., 1989]. The prevalence is quite high in Japan [Hayashi et al., 1995], Egypt, and sub-Saharan Africa [Coursaget et al., 1990; Delaporte et al., 1993]. Numerous HCV isolates from different geographical origins have been sequenced partially or completely; nucleotide sequence comparison and variation of the deduced amino acid sequences reveal that the HCV genome displays a rather high heterogeneity both among infected individuals [Enomoto et al., 1990] and within a single individual [Tanaka et al., 1992]. Several authors have proposed systems of nomenclature of HCV genotypes [Enomoto et al., 1990; Nakao et al., 1991; Chan et al., 1992; Okamoto et al., 1992; Simmonds et al., 1994b]. Recently, efforts have been made to find an acceptable unified system of nomenclature and Simmonds et al. [1994a] have proposed a system based on the nucleotide sequence divergence within the NS5 region, since sequence analysis of the entire genomes of many isolates

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TABLE I. Primers Used for PCR Amplifications

Primer	Sequence (5′ to 3′)	Position[a]	Reference
5′UTR			
939 (+)[b]	CTGTGAGGAACTACTGTCTT	−297	Okamoto et al. [1990]
209 (−)	ATACTCGAGGTGCACGGTCTACGAGACCT	8	Garson et al. [1990]
940 (+)	TTCACGCAGAAAGCGTCTAG	−278	Okamoto et al. [1990]
211 (−)	CACTCTCGAGCACCCTATCAGGCAGT	−29	Garson et al. [1990]
Core			
CPS14 (+)	GCGGAATTCGTGGTACTGCCTGATAGGGTG	−67	Xu et al. [1994]
CPA14 (−)	GGCGGATCCGGAAGATAGAGAAAGAGCAACC	511	Xu et al. [1994]
CPS15 (+)	GGCGAATTCTCTCGTAGACCGTGCACCATG	−26	Xu et al. [1994]
CPA15 (−)	GCTAGATCTTCCCTGTTGCATAGTTCACGCCG	480	Xu et al. [1994]
NS5			
1203 (+)	ATGGGGTTCTCGTATGATACCCGCTGCTTTGACTC	7903	Mellor et al. [1995]
1204 (−)	GGAGGGGCGGAATACCTGGTCATAGCCTCCGTGAA	8275	Mellor et al. [1995]
518 (+)	CTCAACSGTCACSGAGARGGCAT	7935	Mellor et al. [1995]
123 (−)	GCTCTCAGGTTCCGCTCGTCCTCC	8250	Mellor et al. [1995]

[a]According to Choo et al. [1991].
[b](+) positive or (−) negative polarity.

TABLE II. Results of RIBA and RT-PCR of 16 Anti-HCV ELISA-Positive Donors*

	RIBA-2					RT-PCR		
Sample	5-1-1	C100-3	C33c	C22-3	Interpretation	5′UTR	NS5	Core
020	−	−	−	−	−	−	NT	NT
041	−	−	−	−	−	−	NT	NT
089	−	−	+	+	+	+	+	+
091	−	−	−	−	−	−	NT	NT
116	+	+	+	+	+	+	−	−
145	−	−	−	−	−	−	NT	NT
155	−	−	−	−	−	−	NT	NT
202	+	+	+	+	+	+	−	−
220	−	−	−	−	−	−	NT	NT
230	−	−	−	−	−	+	+	−
231	−	−	−	−	−	−	NT	NT
274	−	−	−	−	−	−	NT	NT
280A	−	−	−	−	−	−	NT	NT
281	+	+	+	+	+	+	+	+
323	−	−	+	−	Indeterminate	−	−	−
329	−	−	+	−	Indeterminate	−	−	−

*Four donors were RIBA-positive and two indeterminate. Five were RT-PCR-positive, including a RIBA-negative sample (230). NT = not tested.

in an unrealistic proposition. This system recognizes degrees of divergence among various HCV isolates. Thus, new isolates with less than 72% nucleotide identity with isolates of known sequence potentially may be considered new genotypes; subtypes within a genotype have 75%–86% identity with one another; and isolates within a subtype have greater than 88% identity [Simmonds et al., 1994a].

There is a suggestion that progression and outcome of HCV disease and response to antiviral therapy such as with interferon may vary with genotype [Kanai et al., 1992; Chan et al., 1993; Chemello et al., 1994]. Furthermore, HCV of diverse genotype may elicit serological responses which are undetectable by commercial assays and also may be undetectable by the polymerase chain reaction (PCR) where sequence variation occurs in the primer binding sites. The presence of diverse genotypes within a population may have implications for diagnostic testing and future vaccine development.

Nucleotide sequences have been determined for HCV isolates from many countries worldwide but relatively few have been reported from sub-Saharan Africa. The majority of these were of genotype 4 [Bukh et al., 1992; Simmonds et al., 1993; Xu et al., 1994; Stuyver et al., 1994]. Moreover, none has been reported from Nigeria. In the present study, a pilot survey of HCV infection was carried out among adult donors and children of preschool age in Nigeria. We aimed to correlate antibody positivity with viremia, as determined by the PCR, and to determine the predominant genotypes by sequence analysis.

MATERIALS AND METHODS

Serum samples from 200 healthy adult blood donors and 100 children of preschool age were screened for anti-HCV using a second generation enzyme-linked immunosorbent assay (ELISA) (Murex Diagnostics, Dartford, UK). The ELISA-positive sera were tested further with second generation recombinant immunoblot assay

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                -250
HCV-1           GGCGTTAGTA TGAGTGTCGT GCAGCCTCCA GGACCCCCCC TCCCGGGAGA GCCATAGTGG
HCV 089         .......... .......T.. A......... .......... .......... ..........
HCV 202         .......... .......T.. A......... .......... .......... ..........
HCV 230         .......... .......... .......... .......... .......... ..........
HCV 116         .......... .......T.. A......... .......... .......... ..........
HCV 281         .......... .......... A......... .......... .......... ..........

                -189
HCV-1           TCTGCGGAAC CGGTGAGTAC ACCGGAATTG CCAGGACGAC CGGGTCCTTT CTTGG*ATCA
HCV 089         .......... .........T. ...*....C. ..G...T... .......... .....A..A.
HCV 202         .......... .......... ........C. ..G....... .......... .....*..T.
HCV 230         .......... .......... .......... .......... .......... .....*....
HCV 116         .......... .........T. ........C. ..G...T... .......... .....A..A.
HCV 281         .......... .......... ...*...... ..G...T... .......... .....A..A.

                -129
HCV-1           ACCCGCTCAA TGCCTGGAGA TTT*GGGCGT GCCCCCGCAA GACTGCTAGC CGAGTAGTGT
HCV 089         .......... ....C...A. ...G...... .......... .......... ..........
HCV 202         .......... ....C...A. ...*...... .......... .......... ..........
HCV 230         .......... .......... ...*...... .......... .......... ..........
HCV 116         .......... ....C..... ...G...... .......... .......... ..........
HCV 281         .......... .......... ...*...... .......... .......... ..........

                -69
HCV-1           TGGGTCGCGA AAGGCC
HCV 089         .......... ......
HCV 202         .......... ......
HCV 230         .......... ......
HCV 116         .......... ......
HCV 281         .......... ......
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Fig. 1. Sequences of cDNA amplified from the 5'UTR of five HCV isolates from adult blood donors in Nigeria. The sequences are aligned with the HCV prototype, genotype 1a, and numbered according to Choo et al. [1991]. Asterisk (*) represents deletion in the sequence compared to HCV-1.

(RIBA-2) (Chiron Corporation, Berkeley, CA) and for HCV RNA using reverse transcriptase (RT)-PCR.

For RT-PCR, RNA was extracted from 100 μl of serum using the guanidinium isothiocyanate method (RNaid Kit, BIO 101, Vista, CA). The pellets were resuspended in 30 μl of diethylpyrocarbonate (DEPC)-treated water and 10 μl was used for cDNA synthesis with Moloney murine leukemia virus RT (Pharmacia, Uppsala, Sweden) using random hexanucleotides as primers. PCRs were carried out using the primers listed in Table I.

For amplification of the 5'UTR, first round PCR was carried out in a 100 μl reaction using external primers 939 and 209 and 50 cycles of 94°C for 1 minute, 50°C for 1 minute, and 72°C for 2.5 minutes with a final extension time of 9.9 minutes. Ten microliters of the first round PCR product was used for the second round, nested PCR with internal primers 940 and 211 for 30 cycles with the same conditions. Both rounds of amplification, and extraction steps included appropriate positive and negative controls.

The nucleocapsid region was amplified by nested PCR using primers described by Xu et al. [1994]. First round PCR was carried out using external primers CPS14 and CPA14 and 50 cycles of 95°C for 1.5 minutes, 42°C for 1.5 minutes, and 72°C for 2.5 minutes with a final extension time of 9.9 minutes. Second round PCR was carried out using internal primers CPS15 and CPA15 and 30 cycles with the same conditions.

PCR of the NS5 region was carried with primers described by Mellor et al. [1995] which was highly conserved among different isolates of HCV. First round PCR was carried out using external primers 1203 and 1204

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                7955
HCV-1           ACATCCGTAC GGAGGAGGCA ATTTACCAAT GTTGTGACCT GGACCCCCAA GCCCGCGTGG
HCV 230         .......... .......... ...CA..... .......... .......... .G....T...
HCV 281         .......... ...A...... .......... A......... ...C....TG A.G...A...
HCV 089         C..A....CA C.GA....G. CATCAGG.CC .AG.GAGAGG TCTAT.AGT. TTGT.ACCTC

                8015
HCV-1           CCATCAAGTC CCTCACTGAG AGGCTTTATG TTGGGGGCCC TCTTACCAAT TCAAGGGGGG
HCV 230         .......... .......... .......... .......... .......... ..........
HCV 281         .......A.. .......... .......... ..C....G.A ........C. .C.AA..T..
HCV 089         ATGCAC.A.A G.AAGGG.GA CCT..GCGGT G.CC.TAGGT G.CGCG.T.G CGGC.TATTC

                8070
HCV-1           AAAACTGCGG CTACCGCAGG TGCCGCGCGA GCGGCGTACT GACAACTAGC TGTGGTAACA
HCV 230         .G.....T.. .......... .......... .......... .......... ..........
HCV 281         .......C.. ........TC .....T..A. ..C.G..G.. ...C...... .....C....
HCV 089         .CC..CAGCT TCGGGAACAC ACTGA..T.C TACCTTA.GG CCAC.GCC.. ACA.AGGG.G

                8130
HCV-1           CCCTCACTTG CTACATCAAG GCCGGGCAG CCTGTCGAGC CGCAGGGCTC CAGGACTGCTA
HCV 230         .......... .......... .......... .......... .......... .G........
HCV 281         ....T..C.. T...C....A ..A.C..G.. .......... ....CA.... .A........
HCV 089         G.GGG....C T.CGGGA.CA CA.T.ACGT. .TACCTT.AG GC..CA..CG .ACAGAG.GC

                8190
HCV-1           CCATGCTCGT GTGTGGCGAC GACTTAGTCG TTATCTGTGA AAGTGCGGGG GTCCAGGAGG
HCV 230         .......... .......... .......... .......C.. G......... ..........
HCV 281         .......... ...C...... ...C.T..T. .C........ ...C.GC... ..........
HCV 089         GGCG.GA.T. AAAGATTGCA CCA.GCTGGT C.GCGGCGAC T.CCTG.TC. TCATGCCT.A

                8250
HCV-1           ACGCGGCGAG CCTGAGAGCC
HCV 230         ...A.CG..A ..........
HCV 281         .......A.. ..........
HCV 089         .A.T.A..GC GTC..CGAGG
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Fig. 2. Nucleotide sequence of the NS5 region from isolates HCV230, HCV281, and HCV089. The sequences are aligned with the HCV prototype, genotype 1a, and numbered according to Choo et al. [1991].

with 50 cycles of 95°C for 1 minute, 44°C for 1 minute, and 72°C for 2.5 minutes with a final extension time of 9.9 minutes. The second round PCR was carried out using internal primers 518 and 123 (and, in one case, a seminested reaction with primers 518 and 1204) and 30 cycles of 95°C for 36 seconds, 45°C for 1.5 minutes, and 68°C for 3 minutes with a final extension time of 9.9 minutes.

PCR products were purified using a QIAGEN DNA extraction kit (QIAGEN GMBH, Hilden, Germany). Two microliters (approximately 10 ng) of the purified PCR product was cloned into the TA cloning vector (Invitrogen, San Diego, CA) and used to transform competent cells (INVaF'). Twenty microliters (approximately 10 ng) of the extracted DNA was denatured in 1 M NaOH, 10 mM EDTA (pH 8.0), and kept at room temperature for 30 minutes. The relaxed plasmid DNA was separated by spinning through sepharose CL6B and kept on ice. This was then annealed to the primer and sequencing reactions were carried out using T7 DNA polymerase (Sequenase version 2.0, United States Biochemical Corporation, Cleveland, OH) according to the manufacturer's instructions. Each cloned amplicon was sequenced on both strands; an average of six templates was sequenced in each case. Individual sequences were compared independently to the EMBL data base using the BLAST alignment search program [Karlin and Altschul, 1990; Altschul et al., 1990].

RESULTS

Sixteen (8%) of the 200 adult blood donors, but none of the 100 preschool children, were anti-HCV-positive by ELISA. Four (25%) of the ELISA-positives were positive on supplemental testing using the second generation RIBA and 2 (12.5%) were indeterminate. Table II shows the results of the RIBA testing and RT-PCR (5'UTR,

TABLE III. Pairwise Comparison of Nine Partial Nucleotide Sequences Derived From the NS5 Region of Isolates of HCV Genotype 1 (Nt 8295–8625 According to Choo et al. [1991])*

	1a			1b			1c		
	M62321	M67463	HCV230[a]	M58335	D90208	M84754	L23446	L23447	HCV281[a]
M62321[b]	100	96	95	82	80	81	84	82	85
M67463[b]		100	94	81	80	80	83	81	84
HCV230[a]			100	78	78	77	84	82	84
M58335[b]				100	92	95	83	81	78
D90208[b]					100	91	76	76	80
M84754[b]						100	75	76	79
L23446[b]							100	95	77
L23447[b]								100	76
HCV281[a]									100

*The values represent percentage nucleotide identity between various representatives of HCV genotypes 1a, 1b, and 1c.
[a]Nigerian HCV isolates.
[b]Data base accession number.

NS5, and nucleocapsid regions) for the 16 anti-HCV-positive adult donors. All four RIBA-positive samples were positive for RT-PCR targeted to the 5′UTR. The two RIBA indeterminate samples were negative by RT-PCR. However, 1 of the 10 RIBA-negative samples was positive using RT-PCR.

5′UTR sequences were determined for the five positive samples. Figure 1 is an alignment of these sequences with that of HCV-1. Nucleotide sequence comparison showed that the isolates can be divided into two groups. The first group consists of the HCV isolates from donors 230 and 281 which have greater than 93% identity to various HCV genotype 1 isolates from the United States, Germany, the United Kingdom, and Canada. The second group is from donors 089, 116, and 202 and have greater than 95% homology with published HCV genotype 4 sequences of sub-Saharan African origin [Bukh et al., 1992; Xu et al., 1994]. None of the sequences has 100% identity with any sequence in the data base.

Amplification of the NS5 and nucleocapsid regions was attempted only for the seven samples which were RIBA-positive or indeterminate or positive using RT-PCR of the 5′UTR. None of the samples could be amplified using the method of Valliammai et al. [1995] and primers derived from the NS5 region described by Enomoto et al. [1990] and Chan et al. [1992]. However, three of these samples (089, 230, and 281), all of which were positive using RT-PCR of the 5′UTR, were amplified following attempts with a variety of primer pairs as described by Mellor et al. [1995]. Nested PCR with primers 518 and 123 (Table I) amplified cDNA from samples 230 and 281, while only seminested PCR with primers 518 and 1204 was successful for sample 089.

The results of the sequence analysis of the NS5 region are shown in Figure 2. Comparison with sequences in the data base confirmed the provisional assignment of genotype made from the 5′UTR sequences. The sequence of isolate 230 has greater than 94% homology with genotype 1a sequences, while that of isolate 281 has only 76–85% homology with the various genotype 1 sequences in the data base. Pairwise comparison of the two sequences with seven representatives of genotypes 1a, 1b, and 1c (Table III) clearly shows that isolate 230 can be classified as genotype 1a, but isolate 281 cannot be assigned to the existing subtypes of genotype 1 according to the criteria of Simmonds et al. [1994a,b] because it has less than 86% nucleotide identity with members of those subgroups. Isolate 281 may, therefore, be considered a novel subtype within genotype 1.

The sequence derived from isolate 089 has greatest similarity (82%) with an HCV genotype 4 sequence originating from sub-Saharan Africa. Pairwise comparison of this sequence with the representatives of the genotypes 4a, 4c, 4d, 4e, 4f, 4g, and 4h (Table IV) confirmed that this isolate cannot be assigned to those subtypes. However, NS5 sequences derived from genotype 4b are not available in the data bases at present.

Only two of the samples (281 and 089) could be amplified using primers from the nucleocapsid region following a repeated attempt with reduction of the annealing temperature from 45 to 42°C. These and the others shown as negative in Table II could not be amplified using a 45°C annealing temperature. Sample volumes were insufficient for a repeated attempt with the latter set except for sample 230, which could not be amplified.

Sequence analysis of the nucleocapsid region further confirmed isolate 281 as genotype 1 and isolate 089 as genotype 4. The alignment of these sequences with HCV-1 and the Gabonese type 4c [Xu et al., 1994] is shown in Figure 3. Table V is a pairwise comparison of the isolate 089 nucleocapsid sequence with representatives of genotypes 4a, 4b, 4c, 4e, and 4f. While subtype to subtype sequence variation in HCV is less for the nucleocapsid region than NS5, it is clear that the 089 sequence is distant from that of the genotype 4b isolate. Considering the NS5 and nucleocapsid sequence data together, it seems that isolate 089 represents a new subtype within genotype 4 in addition to the eight subtypes described previously [Stuyver et al., 1994; Mellor et al., 1995]. We have designated this provisionally genotype 4i.

DISCUSSION

A small-scale survey of HCV infection in Nigeria was carried out on adult blood donors and children of preschool age. It should be noted that the donor population

TABLE IV. Pairwise Comparison of 14 Partial Nucleotide Sequences Derived From the NS5 Region of Isolates of HCV Genotype 4*

	L23470	L29602	L29605	EUYEM2	L29590	L29626	L29596	L29618	L29621	L29613	L29611	HCV089	L36438	L36437
L23470[a]	100	83	83	79	86	84	80	78	77	80	79	80	79	82
L29602[a]		100	93	76	85	85	82	78	77	81	80	79	80	81
L29605[a]			100	81	86	82	82	79	79	82	82	79	81	81
EUYEM2[a]				100	84	83	82	77	76	81	80	76	81	79
L29590[a]					100	93	84	80	80	86	86	82	83	83
L29626[a]						100	85	80	80	86	85	82	81	83
L29596[a]							100	81	80	84	83	79	81	81
L29618[a]								100	99	84	84	76	81	76
L29621[a]									100	84	84	76	80	76
L29613[a]										100	99	80	81	81
L29611[a]											100	80	80	81
HCV089[b]												100	81	80
L36438[a]													100	79
L36437[a]														100

*The values represent percentage nucleotide identity with genotypes 4a (L23470), 4c (L29602, L29605), 4d (EUYEM2), 4e (L29590, L29626), 4f (L29596), 4g (L29618, L29621), 4h (L29613, L29611), and two unassigned sequences CAR1 (L36438) and CAR4 (L36437). Pairwise comparisons are made over 329 nucleotides (nt 8276–8605 according to Choo et al., 1991) except for L23470, where only 222 nucleotides are available in the data base.

[a]Data base accession number; EUYEM2 is from Mellor et al. [1995].

[b]Nigerian HCV isolate.

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          -20
HCV-1     TCTCGTAGAC CGTGCACCAT GAGCACGAAT CCTAAACCTC AAAAAAAAAA CAAACGTAAC
HC-G6     .......... .......... .......... .......... ...G.....C ..........
HCV89     .......... .......... .......... .......... ...G.....C ..........
HCV281    .......... .......... .......... .......... ...G.....C ..........

          31
HCV-1     ACCAACCGTC GCCCACAGGA CGTCAAGTTC CCGGGTGGCG GTCAGATCGT TGGTGGAGTT
HC-G6     ........C. ....CAT... ...T...... ......C.T. .C........ ..........
HCV089    ........C. ....CAT... ...T...... ........T. .C........ ...C......
HCV281    ........C. ....CAT... ...T...... ......C.T. .......... ...C......

          131
HCV-1     TACTTGTTGC CGGCAGGGG CCTAGATTGGG TGTGCGCGCG ACGAGAAAGA CTTCCGAGCG
HC-G6     .......... ......... ..C..G..... .......... ...T.G.... ....G.....
HCV089    .......... .......... ..C....... .......... ...TC.G... .....G....
HCV281    .......... .......... ..C..G.... .......... ......G... ..........

          181
HCV-1     GTCGCAACCT CGAGGTAGAC GTCAGCCTAT ATCCCCAAGG CTCGTCGGCC CGAGGGCAGG
HC-G6     .......... ..T..G.... .......... .......... ...A....AT .T.....A..
HCV89     .......... .......... T..T...... .T..AG.C.. .C..A..... ..........
HC281     .......... ..T..A...G .G........ .....C.... ..A..C.... ..........

          231
HCV-1     ACCTGGGCTC GGGATGGCTC CTGTCTCCCC GTGGCTCGGC CTAGCTGGGG CCCCACAGAC
HC-6      T......... .......... .....A.... .T...T..C. T........T ..........
HCV89     ..G.T.G... .......... T........G .......... .......... ..........
HCV281    T......... ..C....... ..C....... .....A.... .A........ .......C..

          291
HCV-1     TACCTCATGG GCCCCCGGCG TAGGTCGCGC AATTTGGGTA AGGTCATCGA TACCCTTACG
HC-G6     ..A....... ......A... .........A ..GTCT.... ....A.AT.. .........A
HCV089    ..A....... .....AG.G. ..C....... ....CT.... ....G.AT.T ..........
HCV281    .....T.... .....T...T .........C .....A.... ..C....... ..........

          361
HCV-1     TGGGCTTCGC CGACCCTTGG CCCCTCTATG GCAGCCCCGG GTAATGAGGG CTGCGGGTGG
HCV-G6    .....C.... .C........ .......... ........A. T.......T. .........T
HCV089    .......... .......... .......... T......... .....C.... .C....A..G
HCV281    .....G.... .T..C...GC .......... ..A....T.. .......T.. ..........

          421
HCV-1     GGTACATACC GCTCGTCGGC GCCCCTCTTG GAGGCGCTGC CAGGGCCCTG GCGCAT
HC-G6     ...A...... ...A....A. .....CG.G. .C....TC.. .......... ..A...
HCV089    ......A... .......... ..T.T..... ....C..G.. ......G.G. ..C.T.
HCV281    ....T..A.. ...G...... ........T. ..C..A.... ..A.T..A.. ..A.A.
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Fig. 3. Sequence of the core region from two Nigerian isolates of HCV (089 and 281) from Nigeria. The sequences are aligned with prototype HCV-1 and Gabonese HCV type 4 (HC-G6) and numbered according to Choo et al. [1991].

in Nigeria predominantly is male. Using a second generation anti-HCV ELISA, a positivity rate of 8% was found for the donors. This is unusually high but the results of supplementary testing and RT-PCR analysis imply a high rate of false positivity. None of the children tested was positive, lending support to the view that mother to infant transmission of HCV is infrequent [Zanetti et al., 1995] and infection probably is acquired later in life through exposure in the community, including percutaneous practices and blood transfusion (although the latter is uncommon in Nigeria).

Through nucleotide sequence analysis from the 5′UTR, NS5, and nucleocapsid regions, combined with pairwise comparisons with HCV sequences in the data bases, we determined that there are at least two major HCV genotypes, 1 and 4, in Nigeria. Genotype 1 is believed to be distributed worldwide and genotype 4 is distributed widely in Africa. Novel subtypes were identifed within both genotypes and these we have designated provisionally 1d and 4i. Genotypes 1 and 4, along with genotype 2, also have been reported from neighboring Cameroon [Ngengasong et al., 1995]. We cannot rule out the possibility of HCV with more divergent sequences and which are not detected by these assays. Failure to amplify the NS5 region of two and the nucleocapsid region of three of the 5′UTR PCR-positive sera may be a further indication of diversity among these viruses.

TABLE V. Pairwise Comparison of Eight Partial Nucleotide Sequences Derived From the Nucleocapsid Region of Isolates of HCV Genotype 4*

	U10236	U10235	U10239	U10238	L29587	L29624	U10240	HCV089
U10236[a]	100	91	89	89	92	93	92	91
U10235[a]		100	89	88	90	91	92	88
U10239[a]			100	95	90	90	92	89
U10238[a]				100	91	92	91	90
L29587[a]					100	96	91	91
L29624[a]						100	91	92
U10240[a]							100	89
HCV089[b]								100

*The values represent percentage nucleotide identity over 463 nucleotides (nt 342–811 according to Choo et al., 1991) and between various representatives of genotypes 4a (U10236), 4b (U10235), 4c (U10239, U10238), 4e (L29587, L29624), and 4f (U10240).
[a]Data base accession number.
[b]Nigerian HCV isolate.

In conclusion, despite the small size of this survey, HCV infection seems to be highly endemic in Nigeria. Detection of HCV in adults but not children of preschool age implies that perinatal transmission is not a major route of infection. There are at least two major genotypes of HCV in Nigeria, genotypes 1 and 4, and novel subtypes are present within these genotypes.

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